

PRODUCT INFORMATION
METHODS OF ANALYSIS

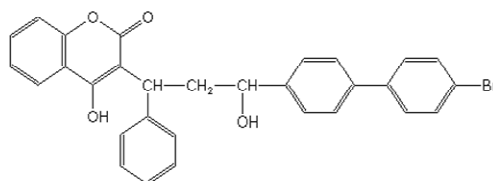
PRODUCT: BRMADIOLONE TECH (ISO, BSI)

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VERSION: 03

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CHEMICAL NAME : 3-[3-(4'-bromo[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-2H-1-benzopyran-2-one



STRUCTURAL FORMULA :

REGISTRATION NO. : PD 20070322(98% Tech) ; LS981281 (0.5% Liquid/Powder)

CAS NUMBER : [28772-56-7]

CHEMICAL FORMULA : $C_{30}H_{23}BrO_4$

MOLECULAR WEIGHT : 527.4

ASSAY BROMADIOLONE TECH

1. OUTLINE:

This sample is dissolved by methanol. With methanol, water, glacial acetic acid as mobile phase, the Bromadiolone in sample is separated and determined by HPLC in stainless steel column with Lichrospher C18 as filling material on wavelength Ultraviolet detector(265nm).

2. REGENTS AND SOLUTIONS

Methanol: reagent (GR)

Glacial acetic acid;

Water: the redistilled water

Brodifacoum standard: known purity $\geq 99.0\%$

3. APPARATUS:

HPLC: Ultraviolet detector with adjustable wavelength;

Chromatography data treater;

Chromatograph Column: 150mm x 4.6 mm(i.d) mm Lichrospher C18 stainless steel column,

Filter: Filter film hole diameter 0.45 μm ;

Micro-Syringe:100 μl ;

Quantitative-sampler: 10 μl ;

Ultrasonic Cleaners.

4. THE OPERATION CONDITION OF CHROMATOGRAPHY

Flow(mobile) phase: (methanol + water + Glacial acetic acid) = 76:24:1 (v/v/v)

Column temperature: room temperature (changes no more than 2 degree)

Wavelength: 265 nm;

Flow rate: 1.0ml/min

Admission valve:10 μl

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Retention time: Bromadiolone A almost 14.09 min, Bromadiolone B almost 15.43 min,
The above chromatograph condition is for the typical operation. To get optimum results, the parameters could be adjusted according to different apparatus.
The typical operation HPLC, the chart 1 & chart 2.

5. DETERMINATION PROCEDURE

Preparation of calibration solution:

Weigh Bromadiolone standard 0.1g (accurate to 0.0001g) into 50ml volume flask. Dissolve with methanol, make up to the mark and shake 3min under ultrasonic for mix thoroughly (A solution). Pippet 5ml (A solution) into 50ml volume flask. Dilute with methanol, make up to the mark and mix thoroughly.

Preparation of sample solution:

Weigh the sample 0.1g (accurate to 0.0001g) into 50ml volume flask. Dissolve with methanol, make up to the mark and shake 3min under ultrasonic for mix thoroughly (A solution). Pippet 5ml (A solution) into 50ml volume flask. Dilute with methanol, make up to the mark and mix thoroughly.

Determination

Under the above operation condition, after stabilized the zero line of apparatus, inject standard solution a couple of time until the variation of the response ratio of the two injection is less than 1.2%, determine by the injection order below: standard solution, sample solution, sample solution, standard solution

Calculation

Sample of Bromadiolone mass percentage X1(%) calculate as formula (1) :

$$X1 = \frac{A2 \times m1 \times P}{A1 \times m2} \quad \text{----- (1)}$$

Where :

A1—average ratio of peak area of Bromadiolone A & B in the standard solution

A2---average ratio of peak area of Bromadiolone A & B in the sample solution

m1—mass of Bromadiolone standard, g

m2—mass of Bromadiolone sample, g

P—mass percent of Bromadiolone in standard, %

Sample of Bromadiolone α (%) calculate as formula (2) :

$$\alpha (A/B) = \frac{AA}{AB} \quad \text{----- (2)}$$

Where :

AA : average ratio of A peak area of Bromadiolone in 2 injection Bromadiolone sample solution

AB : average ratio of B peak area of Bromadiolone in 2 injection Bromadiolone sample solution

Allowable deviation

The deviation of result of parallel determination two times: Bromadiolone is no more than 1.2%